Acta Neuropathol (1995) 89:527–531

REGULAR PAPER

Michael P. Pender · Zsuzsanna Tabi · Kim B. Nguyen
Pamela A. McCombe

The proximal peripheral nervous system is a major site of demyelination in experimental autoimmune encephalomyelitis induced in the Lewis rat by a myelin basic protein-specific T cell clone

Received: 29 November 1994 / Revised, accepted: 18 January 1995

Abstract Experimental autoimmune encephalomyelitis (EAE) was induced in the Lewis rat by the passive transfer of a cytotoxic CD4+ T cell clone specific for the 72–89 peptide of guinea-pig myelin basic protein (MBP). Histological studies on rats with neurological signs showed that inflammation was present in the proximal peripheral nervous system (PNS), namely the spinal roots, as well as in the central nervous system (CNS). The main sites of demyelination were the spinal roots in the PNS, and the spinal cord root entry and exit zones in the CNS. The major involvement of the proximal PNS in autoimmune disease directed at MBP is in marked contrast to EAE induced by immunisation with myelin proteolipid protein, where the inflammation and demyelination are restricted to the CNS. These findings may have implications for the human inflammatory demyelinating diseases including multiple sclerosis, in which MBP is a putative target antigen.

Key words Experimental autoimmune encephalomyelitis · Multiple sclerosis · Myelin basic protein · Polyradiculitis · T cell clone

Introduction

Experimental autoimmune encephalomyelitis (EAE) can be induced in the Lewis rat by active immunisation with whole central nervous system (CNS) tissue, myelin basic protein (MBP) or myelin proteolipid protein (PLP), or by the passive transfer of T cells sensitised to these antigens. Furthermore, CD4+ T cell clones specific for peptide 72–84 [5] or peptide 87–99 [13] of guinea-pig MBP can also induce EAE in this strain. We have previously shown that in EAE induced by active immunisation with MBP or the passive transfer of MBP-sensitised spleen cells there is inflammation and primary demyelination in the proximal peripheral nervous system (PNS) as well as in the CNS [16, 17, 19], whereas in EAE induced by inoculation with PLP the inflammation and demyelination are restricted to the CNS [4]. The present histological study was undertaken to determine the distribution of inflammation and demyelination in EAE mediated by a cytotoxic CD4+ Vβ8.2+ T cell clone specific for guinea-pig MBP peptide 72–89.

M. P. Pender (✉) · Z. Tabi · K. B. Nguyen · P. A. McCombe
Neuroimmunology Research Unit, Department of Medicine, The University of Queensland, Clinical Sciences Building, Royal Brisbane Hospital, Queensland 4029, Australia
Tel.: 61-7-3655132; Fax: 61-7-3655462

Materials and methods

Animals

Lewis rats (JC strain) were obtained from the Central Animal Breeding House of the University of Queensland. The "Principles of laboratory animal care" (NIH publication no. 86-23, revised 1985) were followed, as was the "Australian code of practice for the care and use of animals for scientific purposes" (NHMRC/CSIRO/AAC).

Induction of EAE by the passive transfer of the EC2 clone

The MBP-specific T cell clone EC2 was used in the present study. We have previously characterised it as CD4+, TCRαβ+, Vβ8.2+, CD44+, CD45RC−, specific for the 72–89 amino acid sequence of the guinea-pig MBP molecule, producing interleukin-2 on stimulation with antigen, and cytotoxic for MBP-pulsed syngeneic concanavalin A blasts [21]. EC2 cells were incubated with 10 μg/ml guinea-pig MBP in the presence of irradiated thymocytes in tissue culture media containing 1% normal rat serum for 72 h. Blast cells were isolated by Ficoll density gradient centrifugation and washed three times in phosphate-buffered saline. The blast cells (5×10^6) in 0.5 ml phosphate-buffered saline were intravenously injected into normal 8-week-old male Lewis rats. The severity of EAE was assessed by grading tail, hindlimb and forelimb weakness each on a scale of 0 (no weakness) to 4 (complete paralysis) as previously described [15]. As controls, normal rats were injected intravenously with 5×10^6 blast cells of a T cell line specific for purified protein derivative (PPD).

Histological studies

Histological studies were performed on 14 rats injected with cells of the encephalitogenic EC2 clone and 2 rats injected with cells of

the PPD-specific T cell line. Under anaesthesia, the rats were perfused through the ascending aorta with 0.9% saline followed by 2.5% glutaraldehyde/2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3–7.4). Specimens of the cerebrum, brain stem, cerebellum, lumbosacral spinal cord, lumbar spinal roots, sacrococcygeal spinal roots and sciatic nerve were removed, immersed in fixative and postfixed with 1% osmium tetroxide in dichromate buffer (Dalton's solution). The specimens of the cerebrum, brain stem and cerebellum were embedded in glycol methacrylate (HistoResin, Reichert Jung), sectioned (1 μm) and stained with cresyl fast violet, as previously described [12]. The specimens of the spinal cord, spinal roots and sciatic nerve were stained en bloc with uranyl acetate, dehydrated in a series of graded ethanol solutions followed by absolute acetone, embedded in epoxy resin, sectioned (0.5 μm) and stained with toluidine blue for light microscopy. Light microscopic sections were examined with a Zeiss Axiophot microscope. The extent of demyelination in each region was graded as previously described [19] by estimating the proportion of myelinated fibres demyelinated in transverse sections examined with a 40×/0.75 Plan-Neofluar objective as follows: 0 = no demyelinated fibres; 1 = < 1%; 2 = 1–10%; 3 = 11–30%; 4 = 31–50%; and 5 = > 50% demyelinated fibres. Ultrathin epoxy sections were stained with lead citrate and examined with a Jeol JEM-1200 EXII electron microscope.

Results

Clinical findings

Typically, rats receiving 5×10^6 cells of the encephalitogenic EC2 clone developed distal tail weakness 4–5 days after transfer. Over the next day the weakness ascended to involve the whole tail and was sometimes accompanied by hindlimb weakness and occasionally by forelimb weakness. Clinical recovery commenced 7 days after transfer and was complete by 10 days after transfer. Rats receiving 5×10^6 cells of the PPD-specific T cell line did not develop any neurological signs.

Histological findings

Preclinical phase

Sections through the spinal cord and sacrococcygeal spinal roots were normal in two rats examined before the onset of neurological signs (2 and 3 days after cell transfer, respectively). In two rats examined 4 days after cell transfer, and before the onset of neurological signs, there were occasional mononuclear cells and polymorphs in the sacrococcygeal spinal roots adjacent to, but outside, the fascicles; however, there was no evidence of parenchymal infiltration, oedema or demyelination in these roots, and the spinal cord was normal.

Clinical phase

Six rats with grade 2–3 tail weakness and three rats with grade 4 tail weakness, grade 3 hindlimb weakness and grade 1 forelimb weakness were examined (5–8 days after cell transfer).

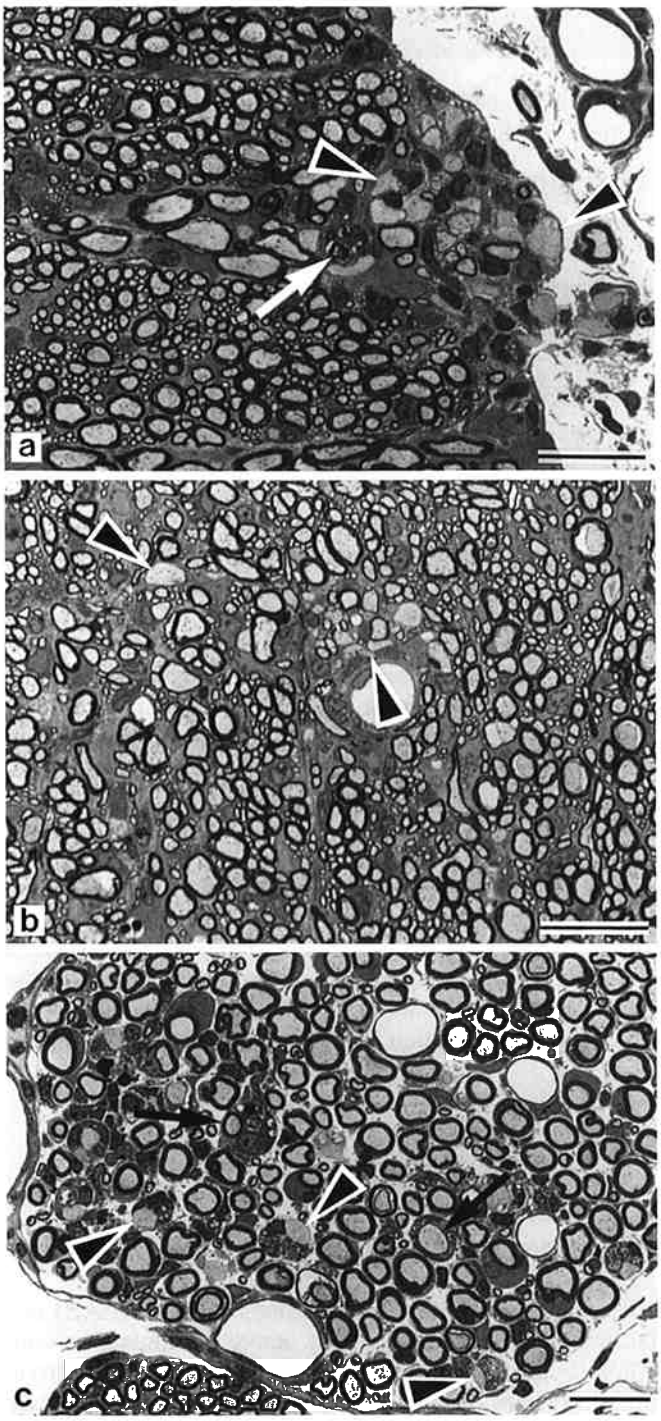

Fig. 1 **a** Light micrograph of the fourth lumbar segment of the spinal cord of a rat with experimental autoimmune encephalomyelitis (EAE) induced by the transfer of encephalitogenic EC2 cells 6 days previously. Demyelinated fibres (*arrowheads*) are present in the CNS portion of the ventral root exit zone. Myelin debris (*white arrow*) can be seen within a macrophage. **b** Light micrograph of the second sacral segment of the spinal cord of the same rat as in **a**. A small number of demyelinated fibres (*arrowheads*) can be seen. **c** Light micrograph of the sacrococcygeal spinal roots of another rat with EAE 6 days after the transfer of EC2 cells, showing fibres in the process of being demyelinated (*arrows*) and numerous completely demyelinated fibres (*arrowheads*). **a–c** Sections (0.5 μm) stained with toluidine blue; *bars* = 25 μm

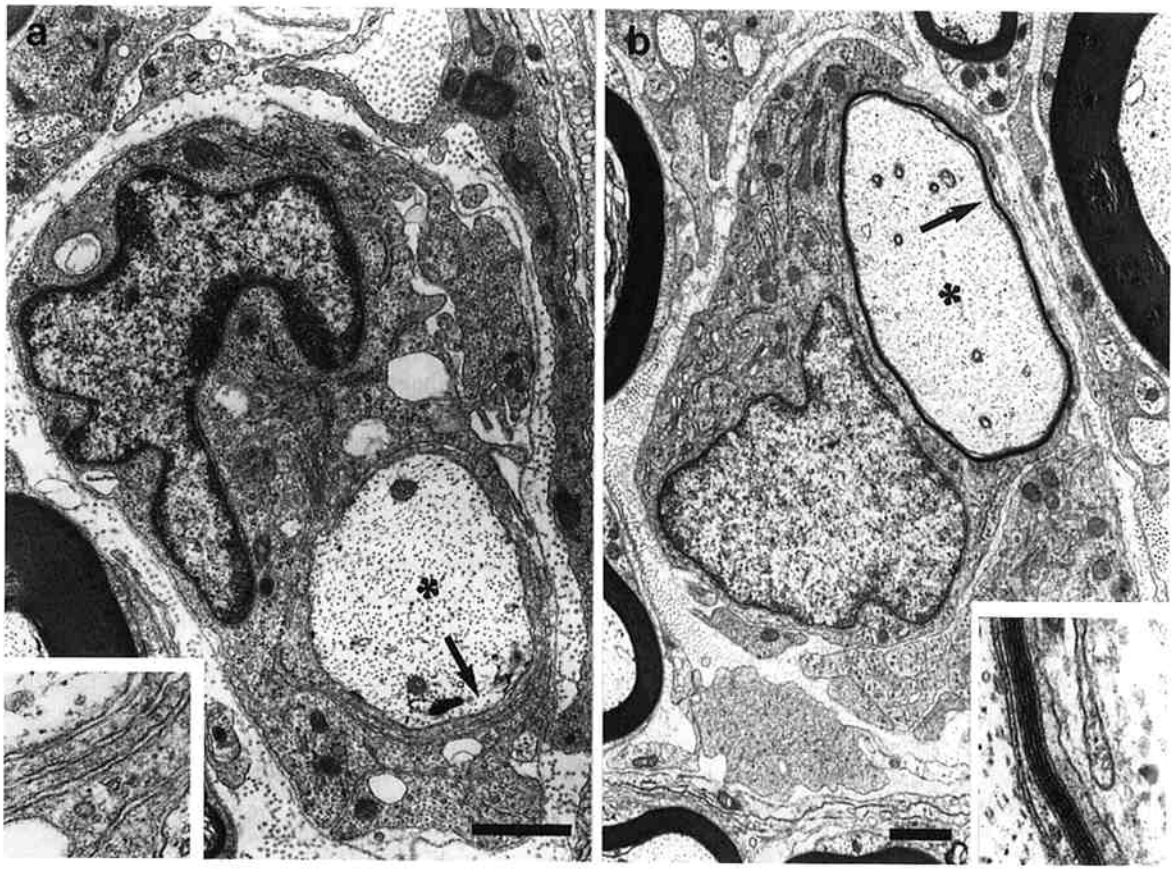

Fig. 2 **a** Electron micrograph of the sacrococcygeal spinal roots of a rat with EAE induced by the transfer of encephalitogenic EC2 cells 8 days previously. A demyelinated axon (*asterisk*) is ensheathed by Schwann cell cytoplasm. *Inset* Higher magnification showing four layers of Schwann cell cytoplasm associated with the axon in the region indicated by the *arrow*. **b** Electron micrograph of the sacrococcygeal spinal roots of a rat that had recovered clinically from EAE induced by the transfer of encephalitogenic EC2 cells 10 days previously. Remyelination of an axon (*asterisk*) with compact myelin can be seen. *Inset* Higher magnification of the region indicated by the *arrow*. **a, b** *Bars* = 1 μm

Central nervous system. At 5 and 6 days after cell transfer, sections through the spinal cord showed prominent meningeal infiltration and perivascular cuffing with mononuclear cells. There was also infiltration of the white and grey matter by mononuclear cells and some polymorphonuclear cells. Apoptotic cells were present in the spinal cord, as we have previously described in other models of EAE [20]. These were mainly present within the parenchyma but were also present in the Virchow-Robin spaces and meninges. They appeared to be mainly lymphocytes or macrophages. Extravasated erythrocytes were also found in the spinal cord parenchyma. There was prominent primary demyelination in the CNS parts of the dorsal root entry zone and ventral root exit zone (Fig. 1a), but demyelinated axons were rare in other regions of the spinal cord (Fig. 1b). Some fibres in the white matter showed dilatation of the periaxonal space within the myelin sheath as described by Brosnan and colleagues [1]. There was minimal or no spinal cord oedema. In the cerebrum, brain stem and cerebellum there was meningeal infiltration, perivascular cuffing and perivascular parenchymal infiltration with mononuclear cells, but neither oedema nor demyelination was observed. This inflammation was limited in the cerebrum and cerebellum but more prominent in the brain stem. At 8 days after transfer, when clinical improvement had commenced, the findings were similar to those at 5 and 6 days after transfer except that the degree of mononuclear cell infiltration was less.

Peripheral nervous system. Sections through the sacrococcygeal spinal roots 5 days after cell transfer revealed parenchymal infiltration by mononuclear and polymorphonuclear cells, oedema and extravasated erythrocytes. Some fibres were in the process of being demyelinated by invading macrophages and some were completely demyelinated (grade 1–2 demyelination). At 6 days after transfer there were numerous fibres in the process of being demyelinated in the sacrococcygeal spinal roots, numerous completely demyelinated fibres (grade 2–3 demyelination) (Fig. 1c), and occasional demyelinated fibres that were ensheathed by a layer of Schwann cell cytoplasm (pro-myelin stage of remyelination). Inflammation and demyelination were also present in the lumbar roots but were less extensive than in the sacrococcygeal roots. At 8 days after transfer there were occasional fibres in the process of being demyelinated and many completely demyelinated fibres (grade 2–3 demyelination), some of

which were ensheathed by one or more layers of Schwann cell cytoplasm (Fig. 2a). Occasional apoptotic cells were present in the spinal roots 6 and 8 days after transfer. These appeared to be mainly lymphocytes or macrophages. There was no evidence of Schwann cell apoptosis. Sections through the dorsal root ganglia showed prominent mononuclear cell infiltration and some demyelination. No lesions were detected in the sciatic nerve.

Recovery phase

One rat was examined after it had completely recovered from grade 3 tail weakness, 10 days after cell transfer.

Central nervous system. There was minimal mononuclear infiltration in the meninges and spinal cord and no spinal cord oedema. Occasional apoptotic cells were observed. Some demyelinated fibres and occasional fibres undergoing remyelination by oligodendrocytes were present in the CNS portion of the ventral root exit zone but there was minimal demyelination or remyelination in the CNS other than in the root entry and exit zones. In the white matter there were rare fibres showing dilatation of the periaxonal space within the myelin sheath. Occasional collapsed myelin figures indicating axonal degeneration were present in the dorsal columns.

Peripheral nervous system. Sections through the sacrococcygeal spinal roots revealed minimal mononuclear infiltration and some oedema. Most demyelinated fibres were ensheathed by Schwann cells and some were thinly remyelinated (Fig. 2b). There were occasional fibres in the process of being demyelinated. Occasional apoptotic cells were also present in the spinal roots. No abnormality was detected in the sciatic nerve.

Controls (6 days after transfer of PPD-specific T cell line)

Sections through the spinal cord and sacrococcygeal spinal roots were normal in two rats 6 days after the transfer of cells from a PPD-specific T cell line. No apoptotic cells were seen in the spinal cord or the spinal roots.

Discussion

In the present study we have shown that there is inflammation in the proximal PNS (the spinal roots) as well as in the CNS in EAE induced in the Lewis rat by a cytotoxic $CD4^+$ $V\beta8.2^+$ T cell clone specific for the 72–89 peptide of guinea-pig MBP. The main sites of demyelination were the spinal roots in the PNS, and the spinal cord root entry and exit zones in the CNS. There was little demyelination in other regions of the CNS despite prominent inflammation. This distribution of inflammation and demyelination in the PNS and CNS is the same as that which we have previously found in EAE induced in the Lewis rat by active sensitisation to MBP (MBP-EAE) [16, 17] or by the passive transfer of MBP-sensitised spleen cells [19]. Conduction block due to the PNS and CNS demyelination could account for the neurological signs observed in the present study, with the PNS involvement (demyelinating polyradiculitis) having a major contribution, as in actively induced MBP-EAE [3, 16, 17]. However, structurally minor, yet functionally significant, changes in the myelin sheath, such as disruption of the paranodal axoglial junctions [9], might also lead to conduction block and, thus, contribute to the neurological signs. Heininger and colleagues [7] have demonstrated conduction abnormalities, which they attributed to demyelination, in the spinal cord and spinal roots in Lewis rats with EAE mediated by an MBP-specific T cell line. The importance of demyelination in the production of neurological signs in EAE is indicated by the finding that the passive transfer of T cells specific for myelin/oligodendrocyte glycoprotein (MOG) results in severe CNS inflammation and disruption of the blood-brain barrier but no demyelination or neurological signs, whereas severe neurological signs develop when CNS demyelination is induced by the co-transfer of anti-MOG antibody and anti-MOG T cells [11].

In the present study there was a good correlation between the neurological signs and the evolution of demyelination, particularly in the PNS. The neurological signs commenced on the day of onset of demyelination, stabilised and began to recover when there was minimal ongoing demyelination and when there was evidence of repair of demyelinated PNS axons by ensheathing Schwann cells, and completely resolved when there was widespread ensheathment and some remyelination by Schwann cells in the PNS and some remyelination by oligodendrocytes in the CNS. Thus, the rapid ensheathment and remyelination of demyelinated PNS and CNS fibres by Schwann cells and oligodendrocytes can account for the clinical recovery, as we have previously shown in EAE induced by active sensitisation to MBP [18] or by the passive transfer of MBP-sensitised spleen cells [19].

The involvement of the PNS in EAE induced by MBP-specific T cells is explained by the fact that the P_1 protein from the PNS is identical to CNS MBP [2, 6]. In the present study the PNS involvement was restricted to the spinal roots and dorsal root ganglia, while the peripheral nerves were spared. This distribution of inflammatory demyelination in the PNS may be due to the reduced blood-nerve barrier in the roots [14] and the almost threefold higher concentration of P_1 (MBP) in the spinal root than in the peripheral nerve in the rat [6]. The prominent involvement of the proximal PNS in MBP-EAE is in marked contrast to the lack of PNS involvement in PLP-EAE, where inflammation, demyelination and nerve conduction abnormalities are restricted to the CNS [3, 4].

The major involvement of the proximal PNS in MBP-EAE has implications for the human inflammatory demyelinating diseases including multiple sclerosis (MS), in which MBP is a putative target antigen. Classically, chronic MS has been regarded as a disease confined to the CNS, al-

though PNS involvement may occur, at least in some cases [22]. Restriction of demyelination to the CNS in MS would indicate that the target antigen is not MBP but an antigen confined to CNS myelin, such as PLP or MOG. Involvement of the PNS as well as the CNS in MS might occur when the immune attack is directed at MBP or other antigens, such as galactocerebroside, which are present in both CNS and PNS myelin. Sensitisation to MBP could account for the PNS disease that occurs in some individuals receiving rabies vaccine containing CNS tissue [8] and in patients with acute MS (Marburg's disease) [10].

In conclusion, the proximal PNS is a major site of demyelination in Lewis rats with EAE mediated by an MBP-specific T cell clone. Although this form of EAE clearly differs from typical MS in the distribution of demyelination responsible for the neurological deficit, the study of the relative resistance of the CNS and the susceptibility of the proximal PNS to demyelination in this model may help to elucidate the immunopathology of autoimmune demyelinating disease of the nervous system.

Acknowledgements This work was supported by a project grant from the National Health and Medical Research Council of Australia (NHMRC). Dr. P. A. McCombe holds a NHMRC R.D. Wright award.

References

1. Brosnan CF, Selmaj K, Raine CS (1988) Hypothesis: a role for tumour necrosis factor in immune-mediated demyelination and its relevance to multiple sclerosis. J Neuroimmunol 18: 87–94
2. Brostoff SW, Eylar EH (1972) The proposed amino acid sequence of the P1 protein of rabbit sciatic nerve myelin. Arch Biochem Biophys 153: 590–598
3. Chalk JB, McCombe PA, Pender MP (1994) Conduction abnormalities are restricted to the central nervous system in experimental autoimmune encephalomyelitis induced by inoculation with proteolipid protein but not with myelin basic protein. Brain 117: 975–986
4. Chalk JB, McCombe PA, Smith R, Pender MP (1994) Clinical and histological findings in proteolipid protein-induced experimental autoimmune encephalomyelitis (EAE) in the Lewis rat. Distribution of demyelination differs from that in EAE induced by other antigens. J Neurol Sci 123: 154–161
5. Chou YK, Vandenbark AA, Jones RE, Hashim G, Offner H (1989) Selection of encephalitogenic rat T-lymphocyte clones recognizing an immunodominant epitope on myelin basic protein. J Neurosci Res 22: 181–187
6. Greenfield S, Brostoff S, Eylar EH, Morell P (1973) Protein composition of myelin of the peripheral nervous system. J Neurochem 20: 1207–1216
7. Heininger K, Fierz W, Schäfer B, Hartung HP, Wehling P, Toyka KV (1989) Electrophysiological investigations in adoptively transferred experimental autoimmune encephalomyelitis in the Lewis rat. Brain 112: 537–552
8. Hemachudha T, Griffin DE, Giffels JJ, Johnson RT, Moser AB, Phanuphak P (1987) Myelin basic protein as an encephalitogen in encephalomyelitis and polyneuritis following rabies vaccination. N Engl J Med 316: 369–374
9. Hirano A, Dembitzer HM (1978) Morphology of normal central myelinated axons. In: Waxman SG (ed) Physiology and pathobiology of axons. Raven Press, New York, pp 65–82
10. Lassmann H (1983) Comparative neuropathology of chronic experimental allergic encephalomyelitis and multiple sclerosis. Schriftenr Neurol 25: 1–135
11. Linington C, Berger T, Perry L, Weerth S, Hinze Selch D, Zhang Y, Lu HC, Lassmann H, Wekerle H (1993) T cells specific for the myelin oligodendrocyte glycoprotein mediate an unusual autoimmune inflammatory response in the central nervous system. Eur J Immunol 23: 1364–1372
12. Nguyen KB, Pender MP (1989) Assessment of demyelination in glycol methacrylate sections: a new protocol for cresyl fast violet staining. Stain Technol 64: 163–167
13. Offner H, Vainiene M, Gold DP, Celnik B, Wang R, Hashim GA, Vandenbark AA (1992) Characterization of the immune response to a secondary encephalitogenic epitope of basic protein in Lewis rats. I. T cell receptor peptide regulation of T cell clones expressing cross-reactive Vβ genes. J Immunol 148: 1706–1711
14. Olsson Y (1968) Topographical differences in the vascular permeability of the peripheral nervous system. Acta Neuropathol (Berl) 10: 26–33
15. Pender MP (1986) Ascending impairment of nociception in rats with experimental allergic encephalomyelitis. J Neurol Sci 75: 317–328
16. Pender MP (1988) The pathophysiology of myelin basic protein-induced acute experimental allergic encephalomyelitis in the Lewis rat. J Neurol Sci 86: 277–289
17. Pender MP (1988) Demyelination of the peripheral nervous system causes neurologic signs in myelin basic protein-induced experimental allergic encephalomyelitis: implications for the etiology of multiple sclerosis. Ann N Y Acad Sci 540: 732–734
18. Pender MP (1989) Recovery from acute experimental allergic encephalomyelitis in the Lewis rat: early restoration of nerve conduction and repair by Schwann cells and oligodendrocytes. Brain 112: 393–416
19. Pender MP, Nguyen KB, Willenborg DO (1989) Demyelination and early remyelination in experimental allergic encephalomyelitis passively transferred with myelin basic protein-sensitized lymphocytes in the Lewis rat. J Neuroimmunol 25: 125–142
20. Pender MP, Nguyen KB, McCombe PA, Kerr JFR (1991) Apoptosis in the nervous system in experimental allergic encephalomyelitis. J Neurol Sci 104: 81–87
21. Tabi Z, McCombe PA, Pender MP (1994) Apoptotic elimination of Vβ8.2$^+$ cells from the central nervous system during recovery from experimental autoimmune encephalomyelitis induced by the passive transfer of Vβ8.2$^+$ encephalitogenic T cells. Eur J Immunol 24: 2609–2617
22. Waxman SG (1993) Peripheral nerve abnormalities in multiple sclerosis. Muscle Nerve 16: 1–5